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Radiation Resistance and Injury of *Yersinia enterocolitica*

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The *D* values of *Yersinia enterocolitica* strains IP134, IP107, and WA, irradiated at 25°C in Trypticase soy broth, ranged from 9.7 to 11.8 krad. When irradiated in ground beef at 25 and −30°C, the *D* value of strain IP107 was 19.5 and 38.8 krad, respectively. Cells suspended in Trypticase soy broth were more sensitive to storage at −20°C than those mixed in ground beef. The percentages of inactivation and of injury (inability to form colonies in the presence of 3.0% NaCl) of cells stored in ground beef for 10 days at −20°C were 70 and 23%, respectively. Prior irradiation did not alter the cell's sensitivity to storage at −20°C, nor did storage at −20°C alter the cell's resistance to irradiation at 25°C. Added NaCl concentrations of up to 4.0% in Trypticase soy agar (TSA) (which contains 0.5% NaCl) had little effect on colony formation at 36°C of unirradiated *Y. enterocolitica*. With added 4.0% NaCl, 79% of the cells formed colonies at 36°C; with 5.0% NaCl added, no colonies were formed. Although 2.5% NaCl added to ground beef did not sensitize *Y. enterocolitica* cells to irradiation, when added to TSA it reduced the number of apparent radiation survivors. Cells uninjured by irradiation formed colonies on TSA when incubated at either 36 or 5°C. More survivors of an exposure to 60 krad were capable of recovery and forming colonies on TSA when incubated at 36°C for 1 day than at 5°C for 14 days. This difference in count was considered a manifestation of injury to certain survivors of irradiation.

Ionizing radiation has been proposed as a means of preserving foods. By exposing foods to low doses (<1 Mrad) of ionizing radiation, one may reduce the number of spoilage microorganisms (11, 13, 16) and thereby extend the shelf life of refrigerated foods (8, 18, 28, 29). Similar doses would also inactivate specific nonspore-forming pathogenic micoorganisms and parasites, thereby improving the hygienic quality of refrigerated fresh foods. A dose of 0.5 Mrad, recommended for the destruction of seven log cycles of *Salmonella* in fresh poultry (9, 17), also reduces the numbers of other nonsporeforming pathogens (e.g., *Staphylococcus* , *Shigella*) and typical spoilage organisms by a factor of at least 10^7 and of *Clostridium* spores by a factor of 10 to 100. This process does leave surviving microflora, and, thus, to inhibit multiplication of the surviving micoorganisms, subsequent refrigeration at 5 to 6°C is required. However, *Yersinia enterocolitica*, a gram-negative microbe infecting humans and isolated from various foods (6, 12, 14, 15), can grow at temperatures as low as 0 to 4°C (14, 15). Therefore, if not eliminated by low doses of irradiation, *Y. enterocolitica* could multiply during refrigeration and present a health hazard.

The purpose of this investigation was to demonstrate the effect of various potential processing conditions on the radiation resistance and recovery of *Y. enterocolitica*.

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MATERIALS AND METHODS

Test organisms. Three clinical strains of *Y. enterocolita* (IP107, IP134, and WA), kindly provided by W. H. Lee, U.S. Department of Agriculture, Beltsville, Md., were maintained on Trypticase soy agar (TSA; Baltimore Biological Laboratory) slants at 5°C. For experiments, cells were grown in Trypticase soy broth (TSB; Baltimore Biological Laboratory) for 24 h at 25°C. It should be noted that both TSA and TSB contain 0.5% NaCl.

Irradiation. Three milliliters (ca. 10^9 cells per ml) of a 24-h TSB culture, or 5 g of radiation-sterilized (2.5 Mrad) ground beef inoculated to contain 10^7 cells per g, was irradiated in air over a dose range of 0 to 60 krad in a ^{60}Co gamma source. The dose rate was 10 krad/min. During irradiation, temperatures were maintained at 25, 5, −10, −20, or −30 ± 5°C by the use of liquid nitrogen.

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Frozen storage. Three-milliliter (ca. 10^9 cells per ml) samples of a 24-h culture in TSB or 5-g samples of ground beef (ca. 10^7 cells per g) were stored at −20°C for 1, 2, 3, 10, 20, and 30 days. Samples were thawed at ambient temperature and treated as indicated.

Recovery. Cell suspensions were appropriately diluted with 0.067 M phosphate buffer, pH 7.5 (6), pour plated with TSA, and enumerated after incubation at 36°C for 24 h or as indicated in specific experiments.

Determination of injury. (i) Sensitivity to NaCl. Uninjured cells were able to form colonies on both TSA and TSA with an additional 2.5% NaCl (total NaCl, 3.0%). However, certain injured cells produced colonies in TSA, but not in TSA with an added 2.5% NaCl. The difference in colony counts with and without the added 2.5% NaCl was a measure of injury.

(ii) Sensitivity to 5°C. Unirradiated cells and cells uninjured by irradiation formed colonies on TSA when incubated at either 36 or 5°C. Certain radiation-injured cells produced colonies at 36°C but not at 5°C, and the difference in colony counts at these two incubation temperatures was a measure of injury.

***D* value.** The *D* value is the radiation dose which reduces the microbial population by one log cycle. *D* values were calculated by fitting a least-squares straight line to the data for logarithms of surviving fractions of the population as functions of dose.

RESULTS AND DISCUSSION

Radiation resistance of three strains of *Y. enterocolitica*. The radiation resistance of gram-negative, foodborne bacteria ranges from the extreme sensitivity exhibited by pseudomonads (27) to the extremely resistant *Moraxella-Acinetobacter* group (30). Gram-negative, foodborne pathogens such as *Salmonella typhimurium* are of intermediate resistance. The *D* values for *Escherichia coli, Staphylococcus aureus, Salmonella enteritidis,* and *Moraxella* spp. in beef at −30°C are 59, 95, 107, and 1,672 krad, respectively (21, 30). The three strains of *Y. enterocolitica,* like the pseudomonads, are among the most radiation sensitive of the foodborne microorganisms, with *D* values in TSB approximating 10 krad (Fig. 1). Since there was little, if any, difference in the radiation resistance of these three strains, only strain IP107 was used in subsequent experiments.

Effect of suspending medium on radiation resistance. The resistance of bacterial cells varies with the composition (2, 5) and with the temperature, water activity, anaerobiosis, and pH of the suspending medium (4). The *D* value of *Y. enterocolitica* irradiated at 25°C in ground beef was approximately twice that of cells irradiated in TSB (Fig. 2).

Effect of frozen state on the radiation resistance of *Y. enterocolitica*. The radiation resistance of vegetative bacteria is usually two to five times greater in the frozen state than in the nonfrozen state (1, 4, 10, 19, 23). More cells

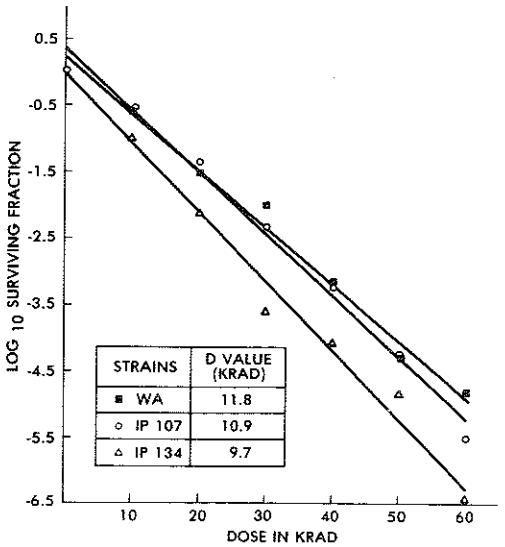

FIG. 1. *Radiation resistance of three strains of Y. enterocolitica in TSB at 25°C. A straight line, not necessarily passing through the origin, was fitted to the data by the method of least squares.*

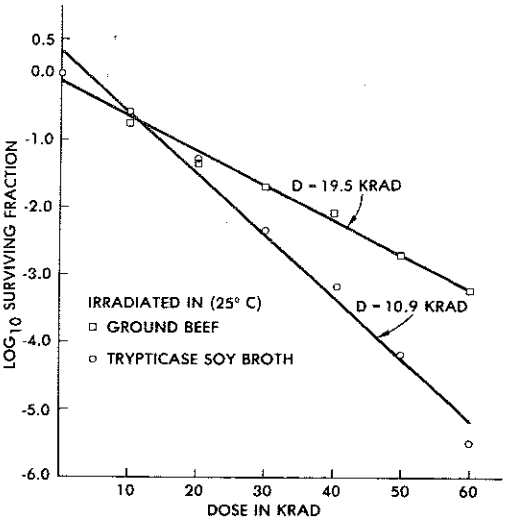

FIG. 2. *Effect of the suspending medium on the radiation resistance of Y. enterocolitica (strain IP107) at 25°C. A straight line was fitted as in Fig. 1.*

of *Salmonella oranienburg,* suspended in chicken meat, were destroyed at an irradiation temperature of 0°C than at −17.7°C (17). Cells of *Y. enterocolitica* in ground beef were twice as resistant when irradiated at −30°C as when irradiated at 25°C (Fig. 3). Furthermore, the major difference in resistance occurred between 5 and −10°C. There was very little difference in resistance between 5 and 25°C or between −10

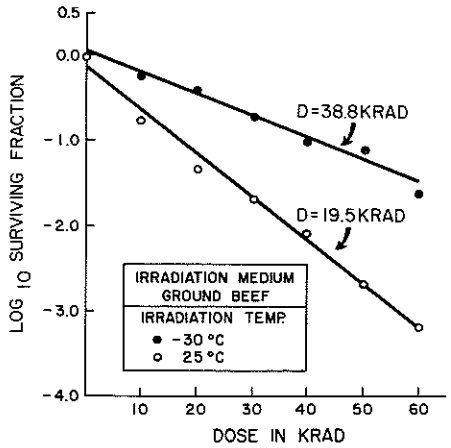

FIG. 3. *Effect of temperature on the radiation resistance of Y. enterocolitica (strain IP107) in ground beef. A straight line was fitted as in Fig. 1.*

and −30°C (data not shown), suggesting that the state (liquid or solid) is more important than the temperature. However, the increased resistance in the frozen state is of little practical significance since most low-dose processing would be of foods in the nonfrozen state.

Effect of NaCl on the radiation resistance and recovery of *Y. enterocolitica*. NaCl up to 4.0%, added to TSA, had little effect on colony formation of *Y. enterocolitica* at 36°C (Fig. 4), 79% as many colonies being formed in TSA with the added 4.0% NaCl as in TSA alone. No colonies were formed in TSA with an added 5.0% NaCl after 10 days of incubation. Okazawa et al. (22) showed that the lethal effect of irradiation on *E. coli* K-12 was enhanced both by the presence of NaCl in phosphate buffer (pH 7.0) during irradiation and in the nutrient agar recovery medium. However, NaCl in an irradiated suspension (distilled water) did not sensitize anaerobic spores to gama irradiation, but when added to an agar recovery medium, it reduced the number of radiation survivors (24, 25). The addition of 2.5% NaCl had little or no effect on the sensitivity to radiation inactivation of *Y. enterocolitica* in TSB (data not shown) or in ground beef (Table 1). The *D* values for cells irradiated in ground beef without and with an added 2.5% NaCl were 19.5 and 18 krad, respectively. Furthermore, the sensitivity of radiation survivors to an added 2.5% NaCl in the recovery medium (TSA) was essentially the same whether the cells were irradiated in the absence or presence of NaCl; i.e., approximately 25% of the radiation (60 krad) survivors in ground beef (with or without an added 2.5% NaCl) were injured (capable of forming colonies in TSA but not in TSA with 2.5% NaCl added). It is conceivable that the mechanism by which NaCl enhances the lethal effect of irradiation on *E. coli* K-12 cells suspended in phosphate buffer (22) was prevented by certain constituents of TSB and ground beef, and, therefore, a similar effect was not evident in our studies with *Y. enterocolitica*.

Effect of incubation temperature on the recovery of irradiated *Y. enterocolitica*. The presence of radiation-injured cells was also evidenced by the inability of some of the radiation survivors to form colonies on TSA at 5°C (Table 2). The cells were irradiated in TSB. More radiation-injured cells were capable of recovery and producing colonies on TSA when incubated at 36°C for 1 day than at 5°C from 1 to 14 days. Approximately 72% of the surviving population recoverable at 36°C was injured and unable to form colonies at 5°C; 71% of the radiation survivors were injured as judged by colony formation in TSA, with or without 2.5% NaCl added. Cells surviving low doses of irradiation are often injured and, therefore, are less capable of competing with uninjured cells. Gamma radiation-injured *E. coli*, *S. typhimurium*, and *Moraxella* sp. showed an increased sensitivity to freezing and thawing and increased susceptibility to lowered water activity and required a higher minimal temperature for growth than did unirradiated cells (20). It is well established that foods which have been treated by various food processing procedures may contain viable but physiologically injured cells (3, 26).

Effect of storage at −20°C on inactivation, injury, and radiation resistance. Hanna et al. (7) showed both inactivation (up to 99.99%) and injury (inability to form colonies in bismuth sulfite agar) of *Y. enterocolitica* cells in beef stored at −18 to −20°C. We also observed a reduction in counts of *Y. enterocolitica* in ground beef stored at −20°C (Table 3), but there

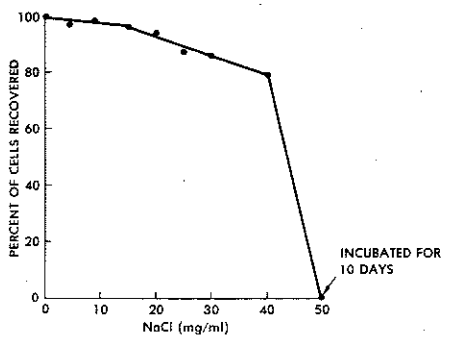

FIG. 4. *Influence of the concentration of NaCl added to the plating medium (TSA) on the recovery of Y. enterocolitica (strain IP107) at an incubation temperature of 36°C. The incubation time was 2 days, except in the case where 5% NaCl was added.*

TABLE 1. *Inactivation and injury of Y. enterocolitica irradiated (60 krad) at 25°C in ground beef with and without 2.5% NaCl*[a]

NaCl added to TSA (%)	Recovery of cells from ground beef with:							
	No NaCl				2.5% NaCl added			
	Unirradiated cells (no./g)	Irradiated cells (no./g)	Inactivation (%)	Injury[b] (%)	Unirradiated cells	Irradiated cells (no./g)	Inactivation (%)	Injury[b] (%)
0	2.7×10^8	1.5×10^5	99.9		1.4×10^8	3.8×10^4	99.9	
2.5	2.6×10^8	1.1×10^5		26.7	1.5×10^8	2.9×10^4		23.7

[a] Colony formation was determined with TSA, with and without an added 2.5% NaCl, after incubation at 36°C for 1 to 2 days.

[b] Injury of survivors was determined by the difference in colony counts of irradiated cells incubated at 36°C on TSA with and without an additional 2.5% NaCl.

TABLE 2. *Influence of incubation temperature on recovery of Y. enterocolitica irradiated (60 krad) at 25°C in TSB*[a]

Incubation temp (°C)	Recovery of:			
	Unirradiated cells (no./ml)	Irradiated cells (no./ml)	Inactivation (%)	Injury (%)
36	2.20×10^9	1.99×10^4	99.9	
5	2.20×10^9	5.50×10^3		72.4[b] (71.0[c])

[a] Colony formation was determined with TSA. Maximum number of colonies was obtained after incubation at 36 and 5°C for 1 and 14 days, respectively.

[b] The difference in colony counts of irradiated cells on TSA incubated at 36 and 5°C was used as a measure of injury.

[c] Injury was estimated as in Table 1, with colony formation at 36°C on TSA ± 2.5% NaCl as the determining factor.

TABLE 3. *Effect of storage period at −20°C on the inactivation and injury of Y. enterocolitica in frozen ground beef and TSB*[a]

Storage period (days)	Ground beef		TSB	
	Inactivation (%)	Injury[b] (%)	Inactivation (%)	Injury[b] (%)
1	0	8.2	67.6	18.5
3	43.0	11.5	85.8	19.0
10	70.4	22.9	99.1	40.8
20	81.5	22.0	99.9	38.9
30	83.3	24.6	99.4	40.1

[a] Frozen ground beef and TSB containing about 10^7 cells per g and about 10^9 cells per ml, respectively, were thawed at ambient temperature for 1 to 2 h and diluted with 0.067 M potassium phosphate buffer (pH 7.6), and cells were incubated on TSA at 36°C.

[b] The difference in colony counts at 36°C on TSA and TSA plus an additional 2.5% NaCl was used as a measure of injury of the surviving population.

was only about 83% inactivation over a 30-day storage period at −20°C. Twenty-four percent of the survivors were injured or unable to form colonies on TSA which contained an additional 2.5% NaCl. Cells suspended in TSB were more sensitive to frozen storage than those in beef (Table 3).

Cells of *Y. enterocolitica* surviving irradiation were no more sensitive to subsequent frozen storage than were nonirradiated cells (data not shown). Furthermore, although some of the nonirradiated cells surviving frozen storage were sensitive to 2.5% NaCl added to TSA, they were no more sensitive to subsequent irradiation at 25°C than were unfrozen cells. Licciardello et al. (17) showed that cells of *S. typhimurium* R12 irradiated in chicken were no more susceptible to frozen storage (−17.8°C) for 12 weeks than were nonirradiated cells.

Y. enterocolitica cells are among the most radiation sensitive of the foodborne bacteria. A dose of 200 krad at 5 to 25°C would reduce *Y. enterocolitica* in meat by 10 log cycles. Furthermore, some cells surviving low doses are injured, as evidenced by their inability to form colonies in the presence of 3.0% NaCl or at an incubation temperature of 5°C. Although cells of *Y. enterocolitica* surviving irradiation do not show an enhanced sensitivity to frozen storage, one can expect a further decrease in counts during storage at −20°C. *Y. enterocolitica* should not be a health hazard in a process of low-dose irradiation.

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